

Does Vascular Calcification Accelerate Inflammation?

A Substudy of the dal-PLAQUE Trial

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ABSTRACT

BACKGROUND Atherosclerosis is an inflammatory condition with calcification apparent late in the disease process. The extent and progression of coronary calcification predict cardiovascular events. Relatively little is known about noncoronary vascular calcification.

OBJECTIVES This study investigated noncoronary vascular calcification and its influence on changes in vascular inflammation.

METHODS A total of 130 participants in the dal-PLAQUE (Safety and efficacy of dalcetrapib on atherosclerotic disease using novel non-invasive multimodality imaging) study underwent fluorodeoxyglucose positron emission tomography/computed tomography at entry and at 6 months. Calcification of the ascending aorta, arch, carotid, and coronary arteries was quantified. Cardiovascular risk factors were related to arterial calcification. The influences of baseline calcification and drug therapy (dalcetrapib vs. placebo) on progression of calcification were determined. Finally, baseline calcification was related to changes in vascular inflammation.

RESULTS Age >65 years old was consistently associated with higher baseline calcium scores. Arch calcification trended to progress more in those with calcification at baseline ($p = 0.055$). There were no significant differences between progression of vascular calcification with dalcetrapib compared to that with placebo. Average carotid target-to-background ratio indexes declined over 6 months if carotid calcium was absent (single hottest slice [$p = 0.037$], mean of maximum target-to-background ratio [$p = 0.010$], and mean most diseased segment [$p < 0.001$]), but did not significantly change if calcification was present at baseline.

CONCLUSIONS Across multiple arterial regions, higher age is consistently associated with higher calcium scores. The presence of vascular calcification at baseline is associated with progressive calcification; in the carotid arteries, calcification appears to influence vascular inflammation. Dalcetrapib therapy did not affect vascular calcification. (J Am Coll Cardiol 2016;67:69-78) © 2016 by the American College of Cardiology Foundation.

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**ABBREVIATIONS
AND ACRONYMS****[¹⁸F]FDG** = ¹⁸F-labeled
fluorodeoxyglucose**ANOVA** = analysis of variance**AU** = Agatston Units**BMI** = body mass index**CHD** = coronary heart disease**HDL** = high-density lipoprotein**LDL** = low-density lipoprotein**MDS** = most diseased segment**PET/CT** = positron emission
tomography/computed
tomography**SHS** = single hottest slice**TBR** = target-to-background
ratio

Atherosclerosis is a chronic, systemic, multifocal inflammatory disorder, a response to the deposition of low-density lipoprotein (LDL) in the vascular wall. Although arterial calcification is thought to be an actively inhibited but passive process of mineralization (1), there is increasing evidence that it is an active and regulated process, analogous to bone formation. That supposition is supported by histological findings of ectopically formed bone, the presence of osteoblast- and osteoclast-like cells, and the secretion of several bone-related peptides within calcified atherosclerotic lesions. In preclinical models, inflammatory macrophage activity is seen to precede early osteogenesis (2).

Vascular calcification serves as a marker for the extent of atherosclerosis and is predictive of cardiovascular events and mortality (3,4). Calcification of the coronary arteries has been extensively investigated. In clinical practice, coronary calcium scoring is used to stratify patients' risk for coronary heart disease (CHD), providing better discrimination than classical risk factors alone (4).

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By comparison, relatively little is known about vascular calcification and its progression in other arterial regions. Contrary to the notion that calcification represents a stable end stage of the disease, dynamic microcalcification may increase the risk of plaque rupture and clinical events (5). Understanding this process is important because rapid progression of calcification, at least in coronary arteries, is associated with an increased risk of cardiovascular events. Current medical therapies, including statins, do not alter this progression (6,7).

In this study, we investigated the vascular calcification detected on serial positron emission tomography/computed tomography (PET/CT) imaging in the dal-PLAQUE (Safety and efficacy of dalcetrapib on atherosclerotic disease using novel non-invasive multimodality imaging) study, a phase 2 randomized clinical trial that investigated the effects of the cholesteryl ester transfer protein inhibitor dalcetrapib on vasculature.

Specifically, we hypothesized that: 1) the presence of classical cardiovascular risk factors would increase both baseline calcification and its progression in the ascending aorta, aortic arch, and carotid and coronary arteries over 6 months; and that 2) arteries with the highest baseline calcium levels would undergo the greatest additional calcification over the next 6 months.

METHODS

The current study was a post-hoc analysis of the dal-PLAQUE study. The study design, methods, and primary results have already been published (8,9). dal-PLAQUE was a phase 2b, double-blind, randomized, placebo-controlled study that investigated the effect of dalcetrapib on vessel wall inflammation, assessed by ¹⁸F-labeled fluorodeoxyglucose ([¹⁸F]FDG) PET/CT. Inclusion criteria included men and women 18 to 75 years of age with known CHD or who were at high risk thereof (with diabetes or a 10-year risk of CHD events >20% by Framingham risk scoring [10]) and whose triglyceride concentrations were ≤400 mg/dl, low-density lipoprotein cholesterol (LDL-C) concentrations were <100 mg/dl, or who were taking maximum tolerated doses of statins and had a target-to-background ratio (TBR) of 1.6 or higher in an index vessel (either right carotid, left carotid, or ascending aorta), as identified by [¹⁸F]FDG uptake measured by PET/CT during the screening period.

PET/CT IMAGING. Details of [¹⁸F]FDG-PET/CT imaging procedures, quantification of tracer uptake, and analyses have been published previously (8). [¹⁸F]FDG-PET/CT imaging of the carotid arteries and ascending aorta was performed at baseline, as well as after 3 and 6 months of follow-up.

Arterial [¹⁸F]FDG uptake was quantified by manually delineating a region of interest on coregistered transaxial PET/CT images. A circular region of interest was drawn to encompass the vessel wall on each contiguous axial segment. Next, the maximum arterial standardized uptake value was determined, defined as the decay-corrected tissue concentration of [¹⁸F]FDG in kBq/ml, adjusted for the injected [¹⁸F]FDG dose and body weight of the patient. We calculated TBR from the ratio of the standard uptake value of the artery compared with that of background

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venous activity and recorded values for the individual vessels (aorta and mean right and left carotid arteries) using previously reported methods (8). The vessel with the highest maximum TBR at baseline was considered the index vessel. Mean of maximum (MeanMax) TBR was the average of the maximal TBR values from each artery. The most diseased segment (MDS) was defined as the average maximum TBR of a group of 3 contiguous slices, centered on the slice with the highest maximum TBR, the single hottest slice (SHS). Active slices were defined as those with TBR >1.6. For the current analyses, we used baseline values and changes at 6 months in the indexes described previously for both the index vessel and the average of both carotid arteries.

ASSESSMENT OF VASCULAR CALCIFICATION. Analysis was performed using baseline and 6-month CT scans by an experienced observer (F.R.J.) blinded to both clinical details and scan order. Unenhanced CT imaging acquired for localization of [¹⁸F]FDG-PET uptake was loaded into the open-source DICOM (Digital Imaging and Communications in Medicine) OsiriX version 4.0 viewer software (OsiriX imaging software, Geneva, Switzerland) and resampled to a 3-mm transaxial thickness.

Using the freely available calcium-scoring plug-in, vascular calcification (on the basis of an attenuation threshold of 130 Hounsfield units in 3 contiguous voxels, after the method of Agatston et al. [11]) was analyzed on consecutive transaxial slices along the length of the arterial segment. The extent of calcification was expressed both as a score in Agatston units and as a volume in cubic millimeters. The measurement of thoracic aortic and coronary artery calcification from ungated CT studies is accurate and highly concordant with values derived from gated studies (12). Paired (baseline and follow-up) studies were visually compared to ensure that the same length of artery was analyzed on both scans.

Coronary artery calcification was scored as the sum of all 3 epicardial vessels. Ascending aortic calcification was scored using transaxial slices from the main pulmonary artery inferiorly to 1 slice below the aortic arch. Aortic arch calcification was scored using transaxial slices from the most inferior slice at which the ascending and descending aorta were contiguous to the origin of the great vessels. Calcification was scored if it appeared to be in the vascular wall. When isolated calcification of the ligamentum arteriosum was obvious, it was not scored. Carotid arterial calcification was assessed bilaterally from the most inferior slice visible on the CT scan to the base of the skull and then summed.

Coronary calcium was not recorded in those patients who had previously undergone percutaneous coronary intervention or coronary artery bypass surgery. If the participant had evidence of prior coronary bypass surgery, the ascending aorta was not analyzed due to artifacts from the surgical clips used in fashioning the grafts. If possible, calcification in the aortic arch was still scored in such cases, provided there were no significant artifacts from sternal wires or surgical clips. Noisy CT images of the neck, with artifacts from dental prostheses, were excluded from analyses of the carotid arteries.

STATISTICAL ANALYSIS. Descriptive data are medians (interquartile range [IQR]) as well as frequencies with proportions for nominal variables, as appropriate.

Measurements of progression of vascular calcification included the absolute differences between baseline and follow-up; the difference between the square root (SQRT) of baseline and SQRT of follow-up score (the SQRT method of Budoff et al. [13]). A cutoff value of 2.5 was used to identify a change in calcification from baseline beyond the known interscan variability of coronary calcium scores, according to Hokanson et al. (14).

Baseline calcium scores in the presence or absence of risk factors were compared after SQRT transformation using analysis of variance (ANOVA). Baseline calcium scores were dichotomized into “zero” and “non-zero,” and the effect of a nonzero score on progression of calcification (absolute change after SQRT transformation) was analyzed using ANOVA. The effect of a nonzero score on absolute changes in PET indices at 3 and 6 months were assessed through linear mixed models with a visit by score interaction. The effect of dalcetrapib treatment on calcium progression (absolute change on original scale and also after SQRT transformation) was analyzed using ANOVA, with baseline and treatment in the model. Two-sided p values are reported throughout, with no adjustment for multiple testing. The lack of adjustment for multiple testing was pre-planned and was stated in our statistical analysis plan for all dal-PLAQUE papers (8,9). Here, we provide raw p values that should be interpreted in the light of multiple testing.

RESULTS

dal-PLAQUE recruited 130 patients with a median 65 years of age, most of whom (82%) were male. Baseline demographics are reported in Table 1. After exclusions, both baseline and 6-month CT images yielded data for the aortic arch in 98 patients (75%),

TABLE 1 Baseline Demographics

	Placebo (n = 66)	Dalcetrapib (n = 64)	Overall (n = 130)
Demographics			
Age, yrs	64.6 (61-71)	62.6 (60-68)	63.6 (60-70)
Males	55 (83)	51 (80)	106 (82)
BMI, kg/m ²	29.8 (25.5-32.3)	29.6 (25.9-32.1)	29.7 (25.7-32.2)
White	62 (94)	58 (91)	120 (92)
Medical history			
Coronary heart disease	54 (82)	57 (89)	111 (87)
Symptomatic coronary artery disease	5 (8)	5 (8)	10 (8)
Hypertension	48 (73)	47 (73)	95 (74)
Type 2 diabetes	20 (30)	19 (30)	39 (30)
Abdominal aortic aneurysm	2 (3)	3 (5)	5 (4)
Peripheral artery disease	10 (15)	6 (9)	16 (12)
Current smoker	8 (12)	9 (14)	17 (13)
Statin use	61 (92)	52 (81)	113 (87)
Imaging parameters at baseline			
Average carotid [¹⁸ F]FDG-PET/CT			
MeanMax TBR	1.76 (1.47-2.10)	1.79 (1.58-2.21)	1.78 (1.50-2.19)
Single hottest slice	1.96 (1.70-2.40)	2.01 (1.83-2.45)	2.00 (1.73-2.44)
Calcium scores, AU			
Ascending aorta	0 (0, 0)	0 (0, 0)	0 (0, 0)
Aortic arch	157 (0-797)	166 (0-865)	161 (0-852)
Coronary arteries	16 (0-89)	10 (0-58)	13 (0-83)
Total carotid arteries	100 (0-315)	27 (1-234)	52 (0-267)
Values are median (interquartile range) or n (%).			
AU = Agatston units; BMI = body mass index; [¹⁸ F]FDG-PET/CT = ¹⁸ F-labeled fluorodeoxyglucose positron emission tomography/computed tomography; MeanMax = mean of maximum; TBR = target-to-background ratio.			

the ascending aorta in 80 patients (62%), the carotid arteries in 102 patients (78%), and the coronary arteries in 32 patients (25%). Data were included only when the whole arterial territory was visualized on both PET and CT scans.

At baseline, the median calcium score in the aortic arch was 161 (IQR: 0 to 852), 52 (IQR: 0 to 267) in the combined carotid arteries, and 13 (IQR: 0 to 83) in the coronary arteries. The median calcium score in the ascending aorta was 0 (IQR: 0 to 0). Given the large number of zero scores (71 of 80 participants), it was not possible to analyze associations between cardiovascular risk factors and baseline scores in the ascending aorta. Across all other arterial territories, baseline calcium scores were higher in those over age 65 years than in younger participants (Table 2). Scores in the aortic arch were significantly higher in those who had ever smoked (329 [IQR: 43 to 1,073] vs. 0 [IQR: 0 to 551], respectively; $p = 0.003$ after SQRT transformation). Calcium scores in the carotid arteries were higher in those with body mass index (BMI) ≤ 29 kg/m² than in those with BMI >29 kg/m² (132 [IQR: 3 to 411] vs. 7 [IQR: 0 to 158], respectively; $p = 0.007$ after SQRT transformation), but not elsewhere. An LDL-C >80 mg/dl was not associated

with significantly higher baseline calcium scores than those with LDL-C ≤ 80 mg/dl. Additionally, high-density lipoprotein cholesterol (HDL-C) concentrations ≤ 40 mg/dl were not significantly associated with higher calcium scores than concentrations >40 mg/dl. A history of CHD, hypertension, type 2 diabetes, peripheral arterial disease, or metabolic syndrome was not associated with higher baseline calcium scores in any territory.

“New” calcification was seldom seen at 6 months in those with no calcium at baseline. This only occurred in 3 of 32 patients (9%) in the coronary circulation and in the carotids in 5 of 102 patients (5%). There was no incident calcification in those with zero baseline scores in either the ascending aorta or the aortic arch (Central Illustration).

Across both treatment groups, progression of SQRT volumes >2.5 was noted in 12 of 32 patients (38%) in the coronary arteries, 18 of 98 patients (18%) in the aortic arch, and 3 of 104 patients (3%) in the carotid arteries.

There was a trend toward greater progression of calcium scores after SQRT transformation in the aortic arch in those with nonzero scores at baseline than in those with zero scores at baseline ($p = 0.055$) (Table 3).

Treatment assignment was not associated with a significant difference in progression of calcium scores in any arterial territory (Table 4; similar data for calcium volumes are not shown).

Comparing differences in absolute change in average carotid PET indexes from baseline to 6 months, TBR in active slices: $p < 0.001$; SHS: $p = 0.006$; MeanMax TBR: $p = 0.003$; and mean MDS: $p = 0.008$ declined if baseline total carotid calcium was zero, relative to those with nonzero scores (Table 5). At 6 months, average carotid PET indexes consistently declined in those with zero total carotid calcium scores (mean active segment TBR: $p < 0.001$; SHS TBR: $p = 0.037$; mean MDS TBR: $p < 0.001$; and MeanMax TBR: $p = 0.010$) (Figure 1). There was no statistically significant change ($p > 0.05$) in these indexes for those with nonzero scores. The presence or absence of calcification elsewhere did not influence changes in measurements of carotid PET uptake. Additionally, there was no significant relationship between baseline calcification and change in measurements of inflammation for the index vessel (ascending aorta in 75% of cases [data not shown]).

DISCUSSION

The study investigated the relationship between risk factors and vascular calcification in several

TABLE 2 Summary of Baseline Calcium Scores by Cardiovascular Risk Factors

		Aortic Arch			Coronary Arteries			Total Carotid Arteries		
		n	Score (AU)	p Value*	n	Score (HU)	p Value*	n	Score (HU)	p Value*
Age, yrs	≤65	53	15 (0-280)	<0.001	17	8 (0-21)	0.038	55	7 (0-100)	<0.001
	>65	45	651 (165-1181)		15	58 (0-204)		48	175 (18-427)	
BMI, kg/m ²	≤29	48	261 (2-941)	0.107	14	10 (0-89)	0.750	50	132 (3-411)	0.007
	>29	50	83 (0-775)		18	19 (0-76)		53	7 (0-158)	
Coronary heart disease	Yes	86	161 (0-797)	0.733	20	13 (0-111)	0.617	90	45 (0-267)	0.611
	No	12	148 (2-860)		12	15 (0-67)		13	68 (0-249)	
Hypertension	Yes	74	165 (0-852)	0.705	24	10 (0-62)	0.078	79	54 (2-276)	0.750
	No	24	57 (2-930)		8	53 (2-301)		24	13 (0-216)	
Type 2 diabetes	Yes	31	200 (0-1022)	0.457	15	4 (0-76)	0.488	32	41 (4-210)	0.565
	No	67	135 (0-775)		17	21 (1-93)		71	54 (0-315)	
Peripheral arterial disease	Yes	13	280 (0-865)	0.249	5	10 (1-204)	0.562	13	100 (29-423)	0.128
	No	85	135 (0-781)		27	16 (0-76)		90	49 (0-249)	
Current smoker	Yes	10	510 (43-1,079)	0.127	3	4 (0-10)	0.262	11	34 (4-173)	0.605
	No	88	118 (0-789)		29	21 (0-89)		92	54 (0-272)	
Ever smoked	Yes	57	329 (43-1,073)	0.003	20	19 (1-109)	0.349	58	69 (4-276)	0.334
	No	41	0 (0-551)		12	5 (0-67)		45	29 (0-224)	
Metabolic syndrome	Yes	53	157 (3-871)	0.650	20	13 (0-62)	0.495	57	25 (0-254)	0.185
	No	45	166 (0-651)		12	29 (1-109)		46	106 (0-267)	
HDL-C, mg/dl	≤40	38	214 (3-1,011)	0.549	11	47 (0-265)	0.279	42	27 (3-315)	0.683
	>40	60	150 (0-753)		21	9 (0-48)		61	66 (0-254)	
LDL-C, mg/dl	≤80	65	100 (0-775)	0.295	19	9 (0-204)	0.391	69	54 (0-276)	0.460
	>80	33	200 (0-1,002)		13	23 (0-48)		34	33 (0-249)	

Values are median (interquartile range). No data are given for the ascending aorta because calcium scores at baseline were zero in 71 of 80 participants. *p values are 2-sided after square root transformation.
 HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; other abbreviations as in Table 1.

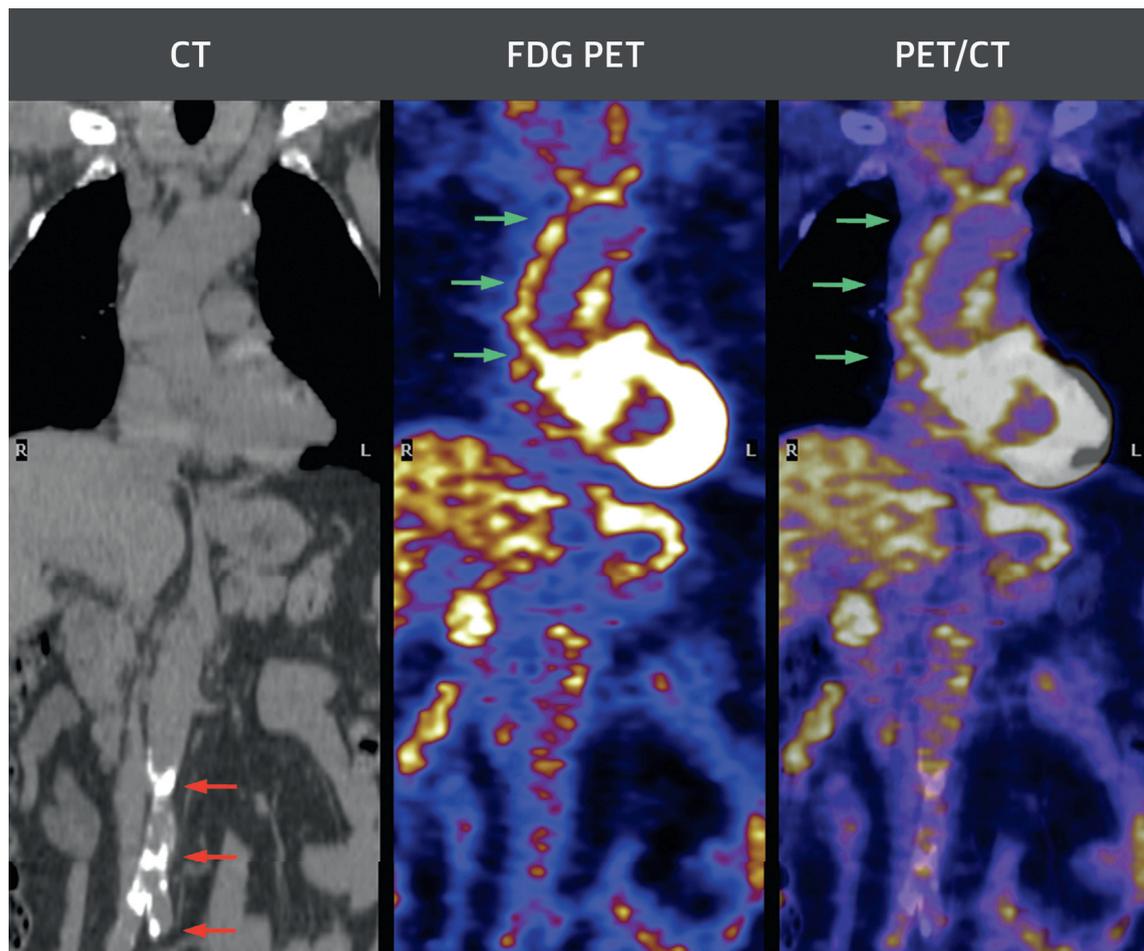
arterial territories and explored the relationship between baseline calcification and changes in inflammation on serial [¹⁸F]FDG-PET/CT imaging (Central Illustration). Whereas previous studies have reported the relationship between arterial calcification and cardiovascular risk factors and progression of calcification in a single arterial territory (usually coronary), the imaging undertaken in the dal-PLAQUE study afforded an opportunity to examine both of these questions. In addition, the first data for progression of aortic calcification outside of renal disease are reported.

Consistently, age >65 years was significantly associated with higher baseline arterial calcification. Generally, the same cardiovascular risk factors influenced atherosclerosis in the coronary, aortic arch, and carotid circulation, as also noted by other investigators (3,15,16). Data from the Rotterdam study found that age and current smoking were the strongest risk factors for calcification in all vessel beds, except for the coronaries. Additionally, hypertension, hypercholesterolemia, and diabetes were independently related to arterial calcification, although associations were not consistent across all vessel beds and for men and women (15). In a study by Rivera et al. (16), increasing thoracic aortic scores were observed with increasing

age. Most traditional cardiovascular risk factors, including age, diabetes, hypertension, smoking, and family history, were significantly associated with the presence of aortic calcification but not LDL-C or HDL-C. Aortic calcification was also associated with incident (new) coronary calcification and played a role in predicting all-cause mortality (17) and cardiovascular events, although not as strongly as coronary artery calcium (18).

An inverse association between high BMI and carotid arterial calcification was noted in our study. Associations between BMI and vascular calcification have generally been inconsistent (19-22), describing positive, inverse, or no association. The “obesity paradox” for cardiovascular risk is well described (23). Although mechanistic insight is lacking, it has been proposed that in females, estrogen production in adipose tissue is relatively protective for the vasculature (24).

Progression of vascular calcification has the potential to better capture the temporal exposure to risk factors compared with a single baseline score. It has been suggested that a baseline calcium score can be thought of as a single point on an atherosclerosis-versus-time curve, whereas progression correlates with the slope of that curve (25). Similarly, although

CENTRAL ILLUSTRATION Calcification Progression and Vascular Inflammation: A Substudy of the dal-PLAQUE Trial

Joshi, F.R. et al. J Am Coll Cardiol. 2016; 67(1):69-78.

Coronal CT, [¹⁸F]FDG-PET, and fused PET/CT images of the thorax and abdomen in a patient with established atherosclerosis are shown. White matter (**left panel CT red arrows**) is calcium within the atherosclerotic abdominal aorta. In the corresponding [¹⁸F]FDG-PET image (**middle panel**), there is little overlap of [¹⁸F]FDG signal in this region, confirmed on the fused image (**right panel**). In contrast, the ascending aorta (**middle and right panels green arrows**) is not calcified, but significant [¹⁸F]FDG uptake is seen. This snapshot illustrates that inflammation and calcification rarely overlap. The present paper extends these observations by highlighting the fact that different cardiovascular risk factors seem to drive vascular inflammation and calcification in the short term. We examined this in multiple vascular beds. Calcification progression over time is rare without calcium at baseline ("calcium begets calcium"), but the same does not apply to vascular inflammation. There was no effect on either pathological process of the HDL-raising therapy dalcetrapib. CT = computed tomography; [¹⁸F]FDG = ¹⁸F-labeled fluorodeoxyglucose; HDL = high-density lipoprotein; PET = positron emission tomography.

baseline scores might reflect atherosclerotic plaque burden, progression might provide insight into ongoing current disease activity. Rapid progression of coronary calcium scores is independently predictive of mortality (13). Our study demonstrated very little newly detectable calcification at 6 months in those without it at baseline, presumably due to the relatively short interval between scans. Nevertheless, our data are consistent with those of Min et al. (26), who

demonstrated that in subjects over 45 years of age, approximately 5% of those without detectable coronary calcium on CT would develop it over 12 months, largely dependent on the number of cardiovascular risk factors present.

In those with some degree of calcium at baseline, there were larger increases in calcium scores within the aortic arch and right carotid arteries than in those with nonzero scores in the same arteries at the start of

the study. To the best of our knowledge, these are the first published data for the progression of aortic calcification outside of the context of end-stage kidney disease or renal transplantation (27).

Van Gils et al. (28) described the progression of carotid calcification in patients after transient ischemic attack or stroke, with 4.7 years between scans. Calcification was measured on contrast-enhanced CT scans using a threshold of 600 Hounsfield units to define calcium. Incident calcification was seen in approximately 40% of those with zero calcium at baseline over the follow-up period and was associated with advanced age and hypertension at baseline. Similarly, the most important determinant of progression of existing calcification was the baseline calcium score, although age, diabetes, hypertension, and smoking were also significantly associated. There are no published data for the prognostic implications of carotid artery calcification. Data regarding its relationship to luminal stenosis are conflicting (29,30).

There were no significant differences in progression of vascular calcification in any arterial territory with dalcetrapib compared to that with placebo. In vitro, HDL-C inhibits osteogenic differentiation and calcification of vascular smooth muscle cells (31). Kuller et al. (32) found an independent association between HDL-C and coronary calcification in postmenopausal females, although there is no evidence for an association with calcification elsewhere (15). Use of [¹⁸F]NaF has been advocated as a means of noninvasively detecting active calcification in vivo (33). ¹⁸F-NaF uptake in the coronary arteries in that study (33) was associated with low HDL-C, suggesting that this might be permissive for progressive calcification. Further research is required to investigate this; to date, however, pharmacological treatment of low HDL-C has not influenced clinical outcomes (34,35).

Over the 6-month interval from first to last PET scan in the dal-PLAQUE study, carotid artery TBR indexes consistently declined in patients without carotid calcification at baseline but remained statistically unchanged when calcification was already present. Calcification in other arterial territories did not appear to influence changes in carotid inflammation, suggesting a local interaction. Inflammation and calcification are thought to be active at different phases of the disease process in atherosclerosis. Inflammation appears to be dominant early, leading to development and maturation of plaques, with calcification predominant later. Pre-clinical models have demonstrated this spatial and temporal relationship (2). Initial deposition of calcium

TABLE 3 Progression in Vascular Calcification Over 6 Months by Baseline (Zero vs. Nonzero) Calcium Score and Volume

	Zero at Baseline		Nonzero at Baseline		p Value*
	n (%)	Median Absolute Change	n (%)	Median Absolute Change	
Calcium scores (AU)					
Ascending aorta	71 (89)	0 (0 to 0)	9 (11)	0 (-10 to 29)	0.900
Aortic arch	29 (30)	0 (0 to 0)	69 (70)	13 (-15 to 138)	0.055
Coronary arteries	11 (34)	0 (0 to 0)	21 (66)	10 (-5 to 68)	0.414
Left carotid	39 (38)	0 (0 to 0)	64 (62)	0 (-11 to 21)	0.774
Right carotid	43 (42)	0 (0 to 0)	59 (58)	5 (-2 to 23)	0.029
Total carotid arteries	28 (27)	0 (0 to 0)	74 (73)	3 (-5 to 33)	0.249
Calcium volumes, mm³					
Ascending aorta	71 (89)	0 (0 to 0)	9 (11)	3 (-11 to 9)	0.536
Aortic arch	29 (30)	0 (0 to 0)	69 (70)	14 (-35 to 121)	0.356
Coronary arteries	11 (34)	0 (0 to 0)	21 (66)	6 (-17 to 187)	0.648
Left carotid	39 (38)	0 (0 to 0)	64 (62)	4 (-8 to 23)	0.995
Right carotid	43 (42)	0 (0 to 0)	59 (58)	8 (-3 to 34)	0.006
Total carotid arteries	28	0 (0 to 0)	74	10 (-8 to 31)	0.225

Values are n (%) and median (interquartile range). *p values are 2-sided after square root transformation.
 AU = Agatston units.

hydroxyapatite, so-called microcalcification, may induce further inflammatory responses (36). Although they are linked, inflammation and calcification are distinct processes within atherosclerosis, and medium-term changes in the relative importance of each in vivo are not well understood. Recent work by Abdelbaky et al. (37) suggests that local vascular inflammation, as assessed by [¹⁸F]FDG-PET, is independently associated with progression of thoracic aortic calcification. In the current study, arterial segments with subsequent incident calcification had the highest baseline [¹⁸F]FDG uptake, suggesting that inflammation precedes calcification. Furthermore, within individual patients, arterial segments with progressive calcification from a nonzero baseline had the highest [¹⁸F]FDG uptake. This suggests that inflammation continues to be important after the development of macroscopic calcification. Segments with calcification that did not progress had the lowest [¹⁸F]FDG uptake. This is consistent with

TABLE 4 Effect of Treatment With Dalcetrapib on Progression of Calcium Scores Over 6 Months

	Absolute Change From Baseline (95% CI)*	p Value	Change from Baseline After SQRT (95% CI)*	p Value
Ascending aorta	-6 (-19 to 7)	0.355	0.17 (-0.73 to 0.38)	0.538
Aortic arch	83 (-4 to 171)	0.061	0.74 (-0.54 to 2.02)	0.254
Coronary arteries	-61 (-171 to 48)	0.263	-1.51 (-4.64 to 1.61)	0.330
Total carotid arteries	6 (-10 to 21)	0.459	0.28 (-0.18 to 0.74)	0.231

Values are differences from placebo with 95% CI; p values are 2-sided. *Absolute change in Agatston units.
 SQRT = square root-transformed values.

TABLE 5 Change in Carotid [¹⁸F]FDG-PET Indices From Baseline to 6 Months for Patients With Zero and Nonzero Baseline Total Carotid Calcium Scores

	Mean Difference (95% CI)	p Value
Mean active segment TBR	-0.23 (-0.35 to -0.11)	<0.001
Single hottest slice TBR	-0.24 (-0.41 to -0.07)	0.006
Mean MDS TBR	-0.18 (-0.31 to -0.05)	0.008
MeanMax TBR	-0.18 (-0.30 to -0.06)	0.003

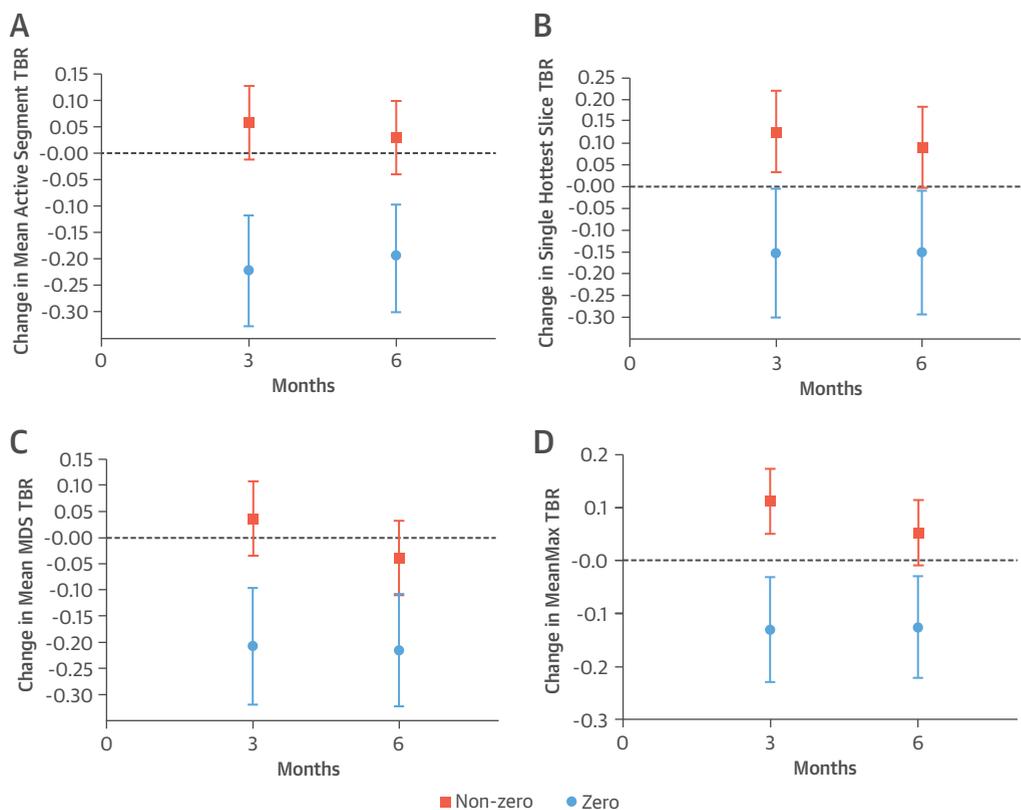
Values are mean differences (zero baseline scores minus nonzero) with 95% CI; 2-sided p values.

[¹⁸F]FDG = ¹⁸F-labeled fluorodeoxyglucose; MDS = most diseased segment; MeanMax = mean of maximum; TBR = target-to-background ratio.

previous work showing that the final stages of calcification are associated with a reduction in inflammatory activity (38). The point at which inflammation becomes less prominent, however, has

yet to be defined. [¹⁸F]NaF uptake holds promise as a means of imaging active calcification in vivo (39), and further longitudinal study of changes in the activity of both inflammation and calcification is needed to better understand these processes.

STUDY LIMITATIONS. This was a post-hoc analysis of data from the dal-PLAQUE study, with only 6 months between PET/CT imaging sessions. Consequently, despite the inclusion of a high-risk population, there was relatively little progression of vascular calcification during this short window. A significant number of scans were excluded from analysis of the coronary arteries because of prior coronary bypass surgery or coronary intervention. Whereas coronary calcium scores on electrocardiogram-gated and ungated CT scans are highly correlated, the inter-scan variability on ungated scans is not known.

FIGURE 1 Vascular Calcification and Vascular Inflammation: Change in Average Carotid [¹⁸F]FDG PET Indexes From Baseline at 3 and 6 Months by Baseline Total Carotid Calcium Score (Zero or Nonzero)

(A) Change in mean active segment (TBR >1.6). Zero calcium score $p \leq 0.001$; nonzero score $p = 0.399$. (B) Change in single hottest slice TBR. Zero calcium score $p = 0.037$; nonzero score $p = 0.061$. (C) Change in mean MDS TBR. Zero calcium score $p < 0.001$; nonzero score $p = 0.285$. (D) Change in MeanMax TBR. Zero calcium score $p = 0.010$; nonzero score $p = 0.095$. Least squares means and 95% confidence intervals are shown for absolute change from baseline. Two-sided p values are given for changes at 6 months from baseline for total (sum left and right) carotid calcium scores were dichotomized into zero (blue) and nonzero (orange). MDS = most diseased segment; MeanMax = mean of maximum; TBR = target-to-background ratio.

Comparison of ungated scans, therefore, may miss small increments in coronary calcium scores, but this is likely to be less of a problem in the other vascular beds studied. Finally, as in any post-hoc data analysis, false positive findings are possible. We advise caution in interpretation of our results, which need to be replicated in longer, larger, prospective studies.

CONCLUSIONS

Inflammation and calcification are important in both the progression of atherosclerosis and its clinical complications. In this study, we have described the relationship between cardiovascular risk factors and both baseline and interval calcification in several arterial beds, as well as the interactions with inflammation in those territories. We have also provided data concerning the drivers of calcification progression and lack of a significant effect of an HDL-raising therapy on calcification. Finally, we have provided the first report of aortic calcification progression outside of the context of subjects with renal failure.

These findings are important for providing a better understanding of the links between these pathological processes and could help to improve patient risk

stratification and provide a platform for developing and testing new atherosclerosis treatments.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: In several vascular territories, evidence of inflammation detected by serial [¹⁸F]FDG-PET/CT imaging appears to precede arterial calcification, and the intensity of [¹⁸F]FDG uptake is associated with the rate of progression of arterial calcification, but temporal relationships vary from one arterial region to another.

TRANSLATIONAL OUTLOOK: Longer-term studies are needed to explore the relationships among vascular inflammation, calcification, and clinical ischemic events.

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